Alveolar fibrocyte percentage is an independent predictor of poor outcome in patients with acute lung injury*

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Objective: **Fibrocytes are mesenchymal progenitors involved in normal and pathologic repair. The aims of this study were: 1) to quantify fibrocytes in bronchoalveolar lavage fluid from patients with or without acute lung injury and acute respiratory distress syndrome; and 2) to evaluate the prognostic value of bronchoalveolar lavage fibrocyte percentage in patients with acute lung injury and acute respiratory distress syndrome.**

Design: **Prospective cohort study.**

Setting: **Three intensive care units of a large tertiary referral center.**

Patients: **One hundred twenty-two ventilated patients requiring bronchoalveolar lavage were enrolled (62 acute respiratory distress syndrome, 30 acute lung injury, 30-ventilated patients without acute lung injury and acute respiratory distress syndrome).**

Interventions: **After bronchoalveolar lavage collection during standard care, the patients were followed up for 28 days and clinical outcome was recorded. Fibrocytes (CD45+/collagen 1+) were quantified in bronchoalveolar lavage by flow cytometry. Comparison of bronchoalveolar lavage fibrocyte percentage from patients with or without acute lung injury and acute respiratory distress syndrome was performed using a Wilcoxon test. A multivariate analysis using a Cox model was performed to study the independent predictors of survival.**

Measurements and Main Results: **Fibrocytes were detected in 90 of 92 (98%) bronchoalveolar lavages from patients with acute lung injury and acute respiratory distress syndrome. The median percentage of bronchoalveolar lavage fibrocytes was significantly higher in patients with acute lung injury and acute respiratory distress syndrome (5.0%) in comparison with ventilated control subjects (0.9%, $p < .0001$). After adjustment for age, comorbidity of malignancy, and severity of illness, a bronchoalveolar lavage fibrocyte percentage >6% was independently associated with a higher 28-day mortality in patients with acute lung injury and acute respiratory distress syndrome (hazard ratio [95% confidence interval] 6.15 [2.78–13.64], $p \leq .0001$). Addition of bronchoalveolar lavage fibrocyte percentage in a clinical model predicting mortality in patients with acute lung injury and acute respiratory distress syndrome improved global fit and discriminatory capacity (c-statistic, 0.78–0.85; $p = .007$).**

Conclusions: **Fibrocytes are detectable in bronchoalveolar lavage during acute lung injury and acute respiratory distress syndrome. A bronchoalveolar lavage fibrocyte percentage >6% provides an additive prognostic value to clinical predictors and may be useful to identify patients with acute lung injury and acute respiratory distress syndrome at highest risk of an adverse outcome. (Crit Care Med 2012; 40:21–28)**

Key Words: bronchoalveolar lavage; collagen 1; fibroblast; fibrosis; progenitor; repair

Acute lung injury (ALI) and its extreme form, acute respiratory distress syndrome (ARDS), remain important causes of mortality in critically ill patients (1, 2). Persistent ARDS is characterized by excessive fibroproliferation, ongoing inflammation, prolonged ventilation, and poor outcome during intensive care unit (ICU) stay (3). The pathobiology of this ineffective repair after ALI is not well understood (4). In experimental models of ALI, bone marrow-derived cells have been shown to be recruited to the lung and to contribute to normal and

***See also p. 300.**

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pathologic repair (5). Among these cells, fibrocytes are mesenchymal progenitors derived from hematopoietic precursors, which coexpress leukocyte (CD45+) and fibroblast markers (collagen 1+) (6). These unique cells are implicated in a wide variety of focal and diffuse remodeling disorders (7), including those localized to the skin, lung, liver, kidney, pancreas, and in atherosclerosis (8). The fibrocytes and their mononuclear precursors (CD14+) (8) are recruited from the blood to organs through multiple chemokine signaling pathways such as CXCL12 (ligand for CXCR4), CCL2 (ligand for CCR2), CCL19 and CCL21 (ligands for CCR7), and CCL3 (ligand for CCR5) (8). In murine models of lung injury, the inhibition of fibrocyte recruitment (9, 10) or the blocking of their differentiation from precursors (11) has been associated with a dramatic limitation of lung fibrosis development. In patients with idiopathic pulmonary fibrosis, the CXCR4/CXCL12 axis has been involved (12, 13) and the detection of an increased number of circulating fibrocytes has been associated with poor outcome (14). In patients with ALI, we recently described that alveolar fibrocytes could be detected in cultures of cells obtained by bronchoalveolar lavage (BAL) (15). On the basis of these observations, we hypothesized that in critically ill patients with ALI, a fibrocyte population is recruited at the site of injury and that their presence could be associated with a poor outcome. The aims of this cohort study, with clinical evaluation 28 days after BAL, were 1) to quantify fibrocytes in BAL during ALI and ARDS and to compare their levels with a control group of ventilated patients without ALI/ARDS criteria; and 2) to examine the prognostic value of BAL fibrocyte percentage in patients with ALI/ARDS.

MATERIALS AND METHODS

Study Population. The protocol was approved by the ethical committee Paris-Ile de France and informed consent was obtained from the patients' relatives. Between June 2006 and April 2008, 122 patients from three ICUs located in Bichat Hospital (two surgical [12 + 12 beds] and one medical [20 beds] ICU, Paris, France) were enrolled consecutively in this prospective observational cohort study. Intubated ventilated patients who required treatment with a BAL during standard care were eligible. Sixty-one patients out of 122 have also been included in a study evaluating the function of alveolar fibroblasts in ALI (15). The BAL was decided by the physician in charge of the patient to confirm clinically suspected ventilator-associated pneumonia (16). Patients with pre-existing fibrotic lung disease, corticosteroid medication, HIV infection, end-stage cancer, age<18 yrs, or current pregnancy were excluded. At the time of inclusion, the ventilated patients were classified into three groups (ALI, ARDS, and a control group without ALI/ARDS) according to the criteria of the American–European Consensus Conference on ALI/ARDS (17). All the patients were ventilated and weaned from the ventilator according to French and European guidelines (18, 19). Positive end-expiratory pressure was individually titrated based on plateau pressure, lung morphology (diffuse vs. focal injury), and on the clinical tolerance (hemodynamic stability, absence of acute cor pulmonale) regardless of the F_{IO_2} level in contrast to the positive end-expiratory pressure/F_{IO_2} scale used in ARDSnet protocol. European and Centers for Disease Control and Prevention guidelines for ventilator-associated pneumonia prevention were used during the present study (20, 21). The day of inclusion, the following clinical data were recorded: age, gender, reason for ICU admission, presence or absence of sepsis and pulmonary infection, PaO_2/F_{IO_2} ratio, $Paco_2$, ventilator setting, Simplified Acute Physiologic Score II on admission to the ICU (22), Sequential Organ Failure Assessment score (23), and Lung Injury Score (24). The length of time between the onset of the mechanical ventilation support and the BAL as well as stay in the ICU were recorded. Patients were followed up for 28 days after inclusion. "Mechanical ventilation duration after BAL" was defined as the number of days under mechanical ventilation after inclusion in this study and censored at 28 days.

BAL Protocol and BAL Fluid Sample Processing. The BAL was performed and processed as previously described (25). A differential BAL cell count was performed on a cytocentrifuge smear with a Diff quik stain kit (Dade International, Miami, FL). BAL fluid (BALF) and plasma protein concentrations were measured with the analyst Hitachi 911 (Roche, Meylan, France), and the BALF/serum protein ratio was determined to evaluate the alveolar permeability induced by lung injury.

Flow Cytometric Analysis. Fibrocytes (CD45+, collagen 1+, CXCR4±) were analyzed by flow cytometry analysis as described in the study by Moeller et al (14) with minor modifications. A part of BAL cell pellet obtained after centrifugation was stored in Cyto-chex storage solution (Streck, La Vista, NE) (1×10^6 cells/mL) for <2 wks at 4°C before analysis. Cells were permeabilized using the Cytofix/Cytoperm kit (BD-Pharmingen, San Diego, CA) and incubated with monoclonal mouse anti-human collagen-1 antibody (Chemicon International, Temecula, CA) or IgG1 isotype control for 30 mins and washed twice, prior labeling with goat antimouse antibody conjugated to fluorescein isothiocyanate (Nordic immunologic Laboratories, Tilburg, The Netherlands). Next, the cells were stained for both surface antigens CD45 (CD45-APC; BD Pharmingen) and CXCR4 (CXCR4-PE; R&D Systems, Minneapolis, MN) for 15 mins. Cells were washed twice and immediately analyzed. Flow cytometry was performed using a FACScalibur flow cytometer (BD Biosciences, Bedford, MA) (Fig. 1). For each sample, at least 10,000 events were collected. Data were analyzed using FlowJo software (Tree Star, Inc., Ashland, OR). The negative threshold for all markers was set using the matched IgG isotype control. Owing to variations in recovered BAL cells among patients with ALI/ARDS, fibrocyte numbers were expressed as a percentage of total BAL cell counts to allow comparisons.

Cytokine, Growth Factors, and Procollagen I Measurements in BALF. C-terminal propeptide of type 1 procollagen was measured in BALF by enzyme immunoassay (OSTEOmedical, Paris, France). The detection threshold of the assay was 0.2 ng/mL; transforming growth factor-β1 (TGF-β1, hepatocyte growth factor, CXCL12, CCL2, CCL19, CCL21, CCL3, and CXCL8 concentrations were measured by enzyme-linked immunosorbent assay according to the manufacturer's recommendations (Quantikine and Duoset Kits; R&D Systems). The detection threshold of the assays were 5 pg/mL for TGF-β1, CXCL12, CCL2, CCL19, CXCL8, and CCL3; 10 pg/mL for CCL21; and 40 pg/mL for hepatocyte growth factor.

Statistical Analysis. The primary objective was to evaluate whether fibrocytes can be detected and quantified in BAL of patients with ALI/ARDS. Forty patients with ALI/ARDS were needed to show that the proportion of patients in which fibrocytes can be detected is >5% with a type 1 error of 5%, a power of 90%, and an expected proportion of patients with ALI/ARDS with fibrocytes in BAL of 30%. Because of the other objectives of the study, it was planned to include 100 patients with ALI/ARDS and 50 ventilated patients without ALI/ARDS. Baseline clinical and BAL characteristics as well as fibrocyte population were compared between patients with or without ALI/ARDS using nonparametric Wilcoxon test or Fisher's exact test for continuous or discrete variables, respectively.

All following analyses were performed in patients with ALI/ARDS. Correlations of fibrocyte percentage to other BAL measurements were assessed with the Spearman correlation test. To investigate the potential confounding of previous duration of mechanical ventilation at the day of BAL collection, we studied by linear regression the link between fibrocyte percentage and duration of ventilation adjusted with severity of patients described by mortality at day 28.

The influence of fibrocyte percentage on survival was then evaluated. A cutoff level for fibrocyte in BAL was determined using the receiver operating characteristics curves for mortality (yes/no) at day 28. The Youden Index

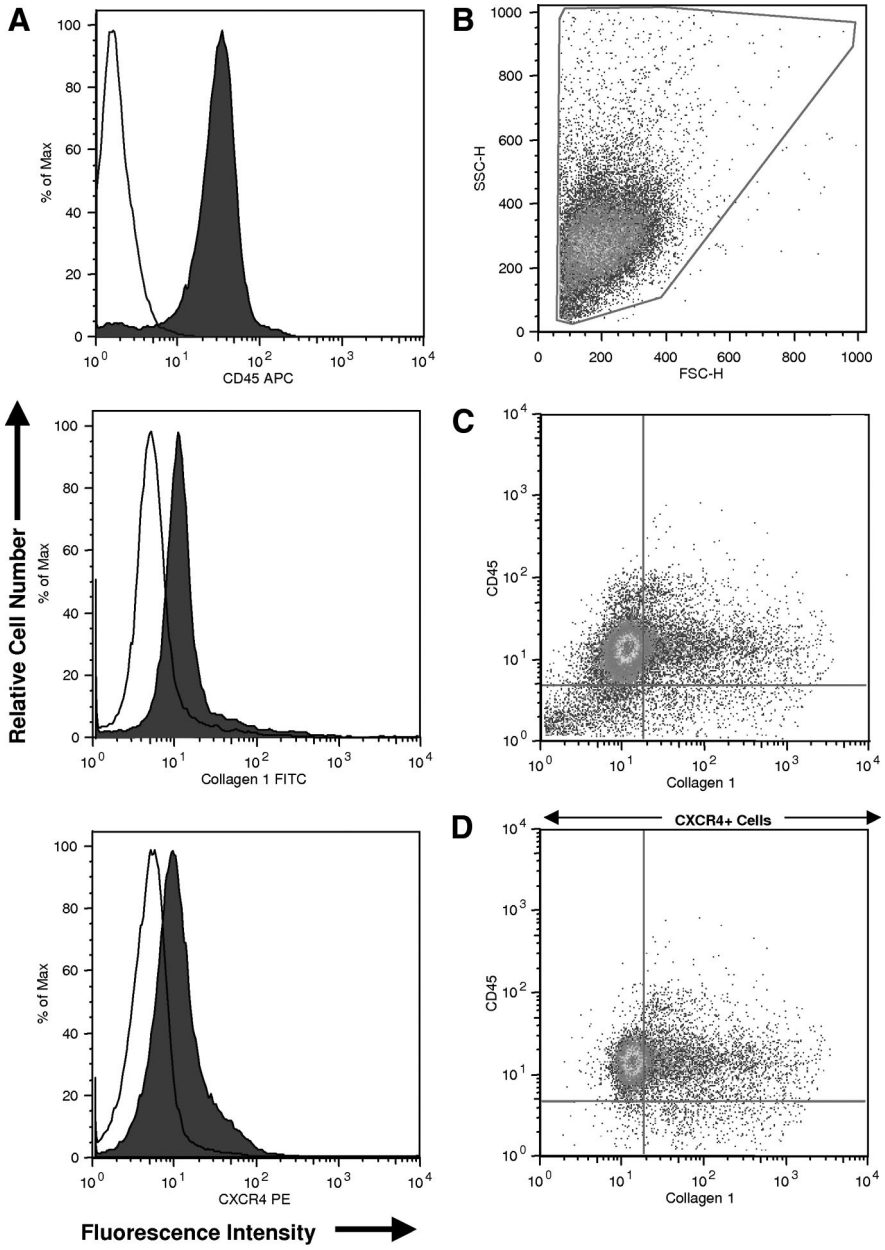

Figure 1. Representative flow cytometric analysis of bronchoalveolar lavage cells from a patient with acute respiratory distress syndrome. *A,* Cells were stained for CD45, collagen 1, and CXCR4 and analyzed by flow cytometry. Isotype control staining of cells is shown as open histograms and CD45, collagen 1, or CXCR4 staining as shaded histograms. *B,* Total cell population selected on unstained cells. *C,* Positive population for CD45 and collagen 1 (*upper right region*). *D,* Positive population for CD45 and collagen 1 among CXCR4+ cells (*upper right region*). Fluorescein isothiocyanate, *FITC*; phycoerythrin, *PE*; allophycocyanin, *APC*; forward scatter, *FSC-H*; side scatter, *SSC-H*.

was used to determine the optimal cutoff point. Kaplan-Meier survival curves were plotted and a log rank test was performed to compare patients above or below the cutoff. A multivariate analysis of the predictors of survival was performed using a Cox proportional hazard model as follows. First, univariate analysis of all clinical and BAL characteristics was performed with fibrocytes in BAL with continuous measurement or using the cutoff. Then, a multivariate analysis was performed starting with a model with all variables with a p value $\leq .20$ in univariate analysis and then performing backward selection to retain in the model only significant variables. C index, a natural extension of area under receiver operating characteristics curve, was used to compare the discrimination ability of several Cox models. Likelihood ratio test and test of differences in C index (using the rcorrp.cens function from Design package of R-software) were performed to compare final Cox models with or without BAL fibrocyte percentage as a covariate.

We also studied duration of mechanical ventilation after BAL in survivors. Kaplan-Meier survival curves were plotted and a log rank test was performed to compare patients above or below the fibrocytes cutoff found in the analysis of mortality. Statistical analyses were performed using R-software, version 2.12.1 (R Foundation, http://www.r-project.org, Auckland, New Zealand) and data presented using GraphPadPrism, version 5.02 (GraphPad Software, LA Jolla, CA).

RESULTS

The planned population necessary to evaluate the primary objective was reached after a 12-month prolongation of the initial time of inclusion in the study. One hundred fifty-one BALs were performed in 132 ventilated patients. Nineteen sequential BALs were not considered in the final analysis. Ten patients were excluded from the study because of HIV infection (n = 1) or noninvasive ventilation (n = 9). We performed the final analysis in a population of 122 patients: 62 patients with ARDS, 30 with ALI, and 30 ventilated patients without ALI/ARDS. The patient characteristics are depicted in Table 1. There was no significant demographic difference among the three groups of patients. The patients in the ALI and ARDS groups were more severely ill than patients without ALI/ARDS as assessed by their severity score (Simplified Acute Physiologic Score II) and their 28-day mortality. At the time of inclusion, the number of organ failures (Sequential Organ Failure Assessment score) as well as the duration of ventilation before the BAL procedure was similar inpatient groups. The patients with ARDS were more severely lung-injured than patients with ALI as demonstrated by higher Lung Injury Score and by a longer duration of mechanical ventilation among survivors. BAL cytology showed a neutrophilic alveolitis in the ALI/ARDS group (Table 2). As expected, the BALF protein concentration and the BALF/serum protein ratio, determined to evaluate the alveolar permeability induced by lung injury, were higher in the ALI/ARDS group than in the control group and depended on the degree of lung injury. We measured a higher levels of inflammatory cytokines (CXCL8, CCL2) and growth factors (hepatocyte growth factor, TGF-β1) in BALF from patients with ALI/ARDS in comparison with ventilated control subjects.

Table 1. Patient characteristics and comparison between patients without acute lung injury/acute respiratory distress syndrome and with acute lung injury/acute respiratory distress syndrome

	Without ALI/ARDS (n = 30)	ALI/ARDS (n = 92)		
		ALI (n = 30)	ARDS (n = 62)	p^b
At admission[a]				
Age, yrs	64 [58–70]	70 [54–77]	67 [49–74]	.69
Male gender	18 (60)	18 (60)	43 (69)	.66
Simplified Acute Physiology Score II score[b]	41 [23–60]	50 [38–69]	48 [39–62]	.03
Comorbiditie(s)				
Smoking	14 (47)	7 (23)	27 (44)	.39
Obstructive airway diseases	6 (20)	5 (17)	11 (18)	.95
Cardiovascular disease	11 (37)	4 (13)	5 (8)	.001
Diabetes	9 (30)	9 (30)	14 (23)	.64
Malignancy	5 (17)	8 (27)	12 (19)	.61
Reason for intensive care unit admission				
Sepsis	4 (13)	10 (33)	17 (27)	.18
Pneumonia	1 (3)	4 (12)	21 (34)	.002
Postoperative	12 (40)	4 (12)	12 (19)	.03
Coma	0 (0)	6 (20)	4 (6)	.01
Congestive heart failure	10 (33)	0 (0)	0 (0)	<.001
Hemorrhagic shock	1 (3)	3 (10)	4 (6)	.58
Other	2 (6)	3 (10)	4 (6)	.82
The day of inclusion[a]				
Sequential Organ Failure Assessment score[c]	7 [5–8]	6 [5–9]	8 [5–10]	.43
Treatment(s)				
Vasopressor	15 (50)	12 (40)	34 (55)	1.00
Antibiotics	18 (60)	17 (57)	39 (63)	1.00
Sedation	29 (97)	27 (90)	60 (97)	1.00
Transfusion	5 (17)	4 (13)	13 (21)	1.00
Insulin	29 (97)	29 (97)	61 (98)	1.00
Heparin	29 (97)	27 (90)	55 (89)	.29
Hemofiltration	3 (10)	4 (13)	10 (16)	.56
Surgical patient	18 (60)	17 (57)	27 (44)	.25
Pulmonary infection	10 (33)	15 (50)	31 (50)	.14
Duration of mechanical ventilation before inclusion, days	4 [1–12]	7 [4–12]	7 [2–12]	.10
Cause of lung injury in ALI/ARDS (n = 92)				
Pneumonia	—	15 (50)	36 (58)	.38
Aspiration	—	3 (10)	7 (11)	1.00
Sepsis	—	11 (40)	15 (24)	.23
Other	—	1 (0)	4 (7)	1.00
Lung Injury Score[d]	1 [0.7–1.7]	1.3 [1–1.7]	2 [2–2.7]	<.001
Respiratory variables				
Minute ventilation, L/min	9.2 [8.1–10.7]	9.5 [7.7–11.2]	9.6 [8.4–11.2]	.51
Respiratory rate, cycles/min	19 [17–20]	20 [18–20]	20 [18–24]	.29
FIO_2	0.4 [0.4–0.5]	0.4 [0.4–0.5]	0.6 [0.5–0.8]	.02
Positive end-expiratory pressure, cm H_2O	5 [5–5]	5 [5–6]	6 [5–8]	.004
PaO_2/FIO_2	247 [190–306]	239 [210–282]	140 [107–167]	<.001
PaO_2, mm Hg	99 [82–149]	102 [86–119]	78 [66–91]	.01
$PaCO_2$, mm Hg	38 [32–42]	41 [34–46]	42 [35–53]	.02
Arterial pH	7.4 [7.34–7.44]	7.4 [7.36–7.42]	7.4 [7.3–7.44]	.68
Outcome parameters[a]				
Mortality 28 days after inclusion	4 (13)	11 (37)	26 (42)	.02
Length of stay in intensive care unit, days	17 [7–42]	25 [14–31]	20 [9–35]	.45

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.

[a]Values are medians and [interquartile ranges] or number and (%). Because of rounding, percentages may not total 100; [b]scores can range from 0 to 163 with higher scores indicating more severe illness; [c]scores can range from 0 to 24 with higher scores indicating more organ failures; [d]scores can range from 0 to 4 with higher scores indicating more severe injury; [e]comparison between patients without ALI/ARDS (n = 30) and ALI/ARDS (n = 92).

Fibrocytes, defined as cells expressing CD45 and collagen 1 (Fig. 1), were detected in BAL by flow cytometry in 90 of 92 (97.8%; 95% confidence interval [CI] 92.4–99.7) of patients with ALI/ARDS and more precisely in 28 of 30 (93.3%) patients with ALI and 62 of 62 (100%) patients with ARDS. Among ALI/ARDS BAL, 48% contained fibrocytes expressing CXCR4. Fibrocytes were detected in 19 of 30 BALs (63.3%) from patients in the group without ALI/ARDS, a proportion significantly lower than in patients with ALI/ARDS ($p < .001$).

The median BAL fibrocyte percentage was significantly higher in patients with ALI and ARDS than in ventilated patients without ALI/ARDS (5.0% vs. 0.93%, respectively, $p < .001$) (Table 2) but was similar among patients with ALI or ARDS (5.0%) (Fig. 2). In patients with ALI/ARDS, the percentage of BAL fibrocytes was positively correlated with percentages of BAL mononuclear cells (s = 0.35, $p = .007$) and no other BAL measurements were significantly associated with fibrocyte percentage. In patients with ALI/ARDS, the BAL fibrocyte percentage was not found to be significantly associated with the duration of mechanical ventilation before BAL, even in a model with adjustment for mortality at day 28, and no significant interaction was found.

Among the patients with ALI/ARDS, 37 died in the 28 days after inclusion and the median BAL fibrocyte percentage in these patients was 2.5-fold higher than in survivors (8.7% vs. 3.4%, respectively; $p < .001$) (Fig. 3*A*). The area under the curve of the receiver operating characteristics curve of fibrocyte percentage for mortality at day 28 was 0.81 (95% CI, 0.73–0.89) (Fig. *3B*). The best Youden index was obtained for a cutoff value of 6% for BAL fibrocyte percentage (Fig. 3*B*). A BAL fibrocyte percentage >6% was detected in 36 patients of 92 (39.1%; 95% CI 29.1–49.9). Kaplan-Meier survival curve corroborated the previous findings; a significant reduction of survival duration was indeed found in patients with a BAL fibrocyte percentage higher than 6% (mean, 14 days; 95% CI 11–17) in comparison with those with a percentage lower or equal to 6% (mean, 25 days 95% CI 23—27; $p < .001$) (Fig. 4). Cox analysis of survival was then performed testing all clinical and biologic parameters reported in Tables 1 and 2. Of note, final Cox models using fibrocyte percentage as a continuous measurement or with the 6% cutoff value were very

Table 2. Characteristics of bronchoalveolar lavage fluid from patients[a]

	Without ALI/ARDS (n = 30)	ALI/ARDS (n = 92)		p^b
		ALI (n = 30)	ARDS (n = 62)	
Bronchoalveolar lavage fluid protein, g/L	0.14 [0.11–0.45]	0.30 [0.20–0.57]	0.36 [0.18–0.49]	0.02
Bronchoalveolar lavage fluid/serum protein ratio	0.002 [0.002–0.009]	0.006 [0.003–0.011]	0.006 [0.003–0.009]	0.05
Total cell count, cells/mm^3	172 [50–676]	461 [89–2652]	327 [100–1471]	0.03
Neutrophil, %	53 [13–90]	79 [63–90]	87 [70–92]	0.004
Mononuclear cells, %	47 [10–87]	21 [10–37]	13 [8–30]	0.004
Fibrocytes, %	0.9 [0–3.4]	5.0 [2.9–9.1]	5.0 [3.2–8.8]	<.001
CXCL8, pg/mL	814 [469–3357]	2433 [1337–5854]	2460 [716–7442]	0.03
CXCL12, pg/mL	<5 [<5–31]	17 [<5–39]	<5 [<5–27]	0.55
CCL2, pg/mL	61 [31–152]	266 [70–475]	147 [40–390]	0.03
CCL3, pg/mL	23 [10–46]	47 [19–124]	35 [10–125]	0.1
CCL19, pg/mL	<5 [<5–29]	12 [<5–58]	6 [<5–37]	0.12
CCL21, pg/mL	<10 [<10–136]	<10 [<10–275]	<10 [<10–278]	0.41
Hepatocyte growth factor, pg/mL	150 [<40–406]	685 [76–1392]	396 [190–1030]	0.001
Transforming growth factor-β1, pg/mL	10 [<5–34]	21 [8–45]	15 [<5–44]	0.08
Procollagen 1, pg/mL	<200 [<200–525]	<200 [<200–2825]	<200 [<200–6013]	0.34

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.
[a]Values are medians and [interquartile ranges]; [b]comparison between patients without ALI/ARDS (n = 30) and ALI/ARDS (n = 92).

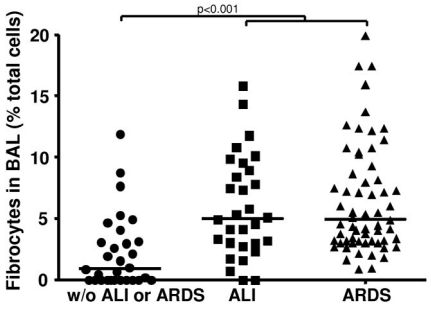

Figure 2. Bronchoalveolar lavage (*BAL*) fibrocyte percentage in ventilated control subjects and patients with acute lung injury (*ALI*) and acute respiratory distress syndrome (*ARDS*). Percentage of fibrocytes (CD45+/collagen 1+) among total cells in bronchoalveolar lavage from ventilated patients without ALI/ARDS (*black circles*, n = 30) and from ventilated patients with ALI (*black squares*, n = 30) or ARDS (*black triangles*, n = 62). *Horizontal black bars* indicate the median of individual values.

close and only the latter are reported here. Cox univariate analysis (Table 3) showed that mortality was associated with age, comorbidity (malignancy), severity at admission (Simplified Acute Physiologic Score II), persistence of organ failure the day of inclusion (Sequential Organ Failure Assessment score, FIO_2, PaO_2, pulmonary infection, vasopressor infusion, transfusion, hemofiltration), and BAL characteristics (fibrocyte and neutrophil percentages, TGF-β1 concentration). After backward selection, the final Cox model found that age, malignancy, Sequential Organ Failure Assessment score, and BAL fibrocyte percentage >6% remained associated with mortality (Table 3). Of note, duration of mechanical ventilation before BAL was not significantly associated with survival in the univariate analysis (hazard ratio 0.97; 95% CI, 0.93–1.01). No significant covariate interaction was found in the final model. The likelihood ratio test to compare the two Cox models with or without fibrocyte percentage was significant ($p < .001$). The overall discrimination C-index in the final Cox model was 0.85 (95% CI, 0.79–0.91), significantly better than the one without fibrocyte percentage (C = 0.78; 95% CI 0.71–0.85; $p = .007$).

Estimation of Kaplan-Meier curves of mechanical ventilation duration after BAL was performed in patients alive at day 28, i.e., eight patients with fibrocytes >6% and 47 patients with fibrocyte <6%. The estimated mean of duration of ventilation after BAL was 9 days (95% CI, 2–17) for patients with BAL fibrocytes >6% vs. 12 days (95% CI 9–15) for those with BAL fibrocytes <6%, and no significant difference was found ($p = .48$).

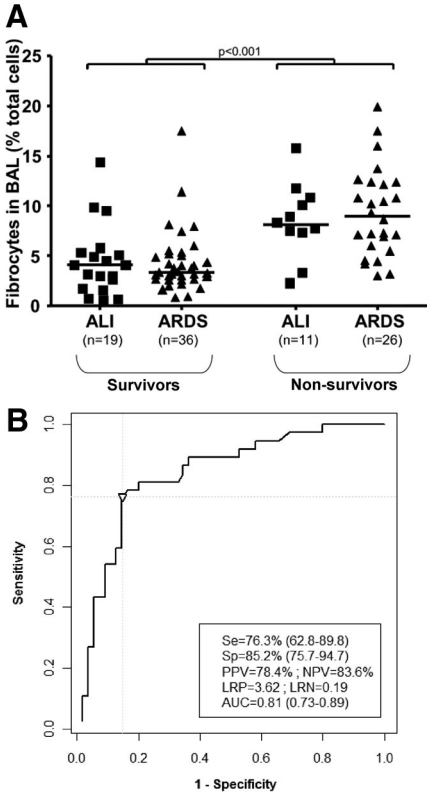

Figure 3. *A,* Comparison of the percentage of fibrocytes in bronchoalveolar lavage (*BAL*) obtained from survivors vs. nonsurvivors of acute lung injury/acute respiratory distress syndrome (*ALI/ARDS*). Percentage of fibrocytes (CD45+/collagen 1+) among total cells in BAL obtained from survivors (n = 55) and nonsurvivors (n = 37) in the ALI (*black squares*) and ARDS groups (*black triangles*). *Horizontal black bars* indicate the median of individual values. *B,* Receiver operating curve curve-based analysis to assess the ability of BAL fibrocyte percentage to predict mortality. Receiver operating curve analysis identified a BAL fibrocyte percentage of 6% as having the highest accuracy as a threshold value for predicting mortality 28 days after the BAL procedure in patients with ALI/ARDS (the cutoff value is symbolized by an *open triangle*). *AUC*, area under the curve; *LRN*, likelihood ratio negative; *LRP*, likelihood ratio positive; *NPV*, negative predictive value; *PPV*, positive predictive value; *Se*, sensitivity; *Sp*, specificity.

DISCUSSION

Our study demonstrates for the first time that 1) fibrocytes can be detected in 98% of BAL from ventilated patients with ALI/ARDS; 2) BAL fibrocyte percentage is significantly increased in ventilated patients with ALI/ARDS as compared with ventilated control subjects; 3) BAL fibrocyte percentage is independently associated with increased mortality; and 4) addition of BAL fibrocyte percentages in a model predicting ALI/ARDS mortality im-

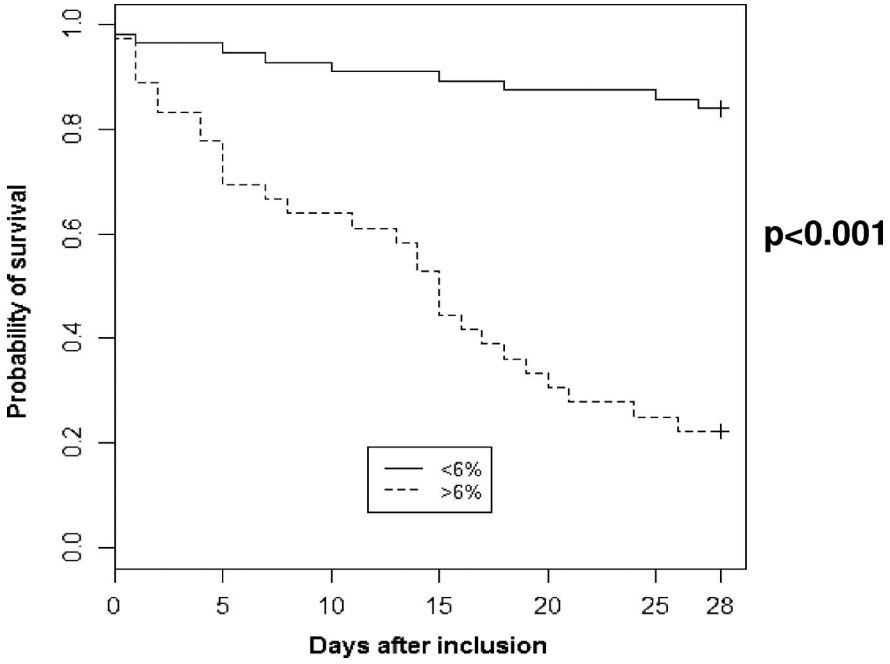

Figure 4. Kaplan-Meier curves of survival in patients with acute lung injury/acute respiratory distress syndrome (n = 92). Patients with >6% fibrocytes (*dotted line*, n = 36) vs. <6% fibrocytes (*solid line*, n = 56).

Table 3. Cox proportional hazard analysis of predictors of mortality in patients with acute lung injury/acute respiratory distress syndrome[a]

	Univariate Cox Model		Multivariate Cox Model	
Variable	Hazard Ratio (95% Confidence Interval)	*p*	Hazard Ratio (95% Confidence Interval)	*p*
At admission				
Age per block of 10 yrs	1.04 [1.11–1.77]	.005	1.37 [1.08–1.75]	.01
Simplified Acute Physiology II score	1.02 [1.00–1.04]	.01	—	—
Comorbidity of malignancy	2.63 [1.33–5.18]	.005	4.14 [1.81–9.47]	<.001
The day of inclusion				
Sequential Organ Failure Assessment score per point	1.23 [1.12–1.35]	<.001	1.27 [1.13–1.44]	<.001
Vasopressor infusion	3.23 [1.59–6.54]	.001	—	—
Transfusion	2.66 [1.33–5.32]	.006	—	—
Hemofiltration	2.86 [1.38–5.93]	.005	—	—
Pulmonary infection	0.49 [0.25–0.96]	.04	—	—
F_{IO_2}	1.02 [1.00–1.03]	.01	—	—
PaO_2, mm Hg	1.01 [1.00–1.02]	.01	—	—
BAL characteristics				
Neutrophils, % in BAL	0.98 [0.97–0.99]	.003	—	—
Percentage of fibrocytes in BAL, >6%	8.20 [3.83–17.6]	<.001	6.15 [2.78–13.64]	<.001
Transforming growth factor-β1, pg/mL BAL fluid	0.99 [0.98–1.00]	.02	—	—

BAL, bronchoalveolar lavage.

[a]Values are medians and [interquartile ranges].

proves the accuracy of clinical and biological models.

Our study is the first to quantify and determine the prognostic value of alveolar fibrocytes in patients with ALI. Several studies have focused on the specific predictive value of serum and BAL mediators in the pathophysiology of ALI/ARDS (26), but none of them has become a standard test to evaluate the prognosis of ALI/ARDS, which remains based on clinical criteria (e.g., age, number of organ failures).

The BAL under fiberoptic bronchoscopy is a common and safe procedure (16, 27) used in ICU to explore the distal lung both for the diagnosis of ventilator-associated pneumonia (16) and to identify noninfectious diseases of the lung (28). The kinetics of fibrocyte recruitment within the lung after an ALI is unknown in humans. In our study, BAL was thus performed only for suspicion of pneumonia, because repeated and systematic BAL analysis was considered unethical in this first human study. Therefore, the patients were sampled during current care after a median time of 6 days under mechanical ventilation (Table 1). Because we did not observe either a correlation or interaction between BAL fibrocyte percentage and time of BAL sampling, our study suggests that BAL fibrocyte percentage remains a valuable indicator of outcome independently of the time of ICU stay.

Based on experimental data, several chemokines including CCL2, CCL3, CCL19, CCL21, and CXCL12 have been involved in the lung recruitment of fibrocytes. In our study, we did not find any significant correlation between the percentage of fibrocytes and BALF chemokine concentrations (CCL2, CCL3, CCL19, CCL21, CXCL12, CXCL8). This result could be explained by different time courses between fibrocyte lung recruitment and local chemokine production, by the lack of chemokine stability in the protease-rich alveolar fluids from patients with ALI/ARDS, and/or by the participation of other mediators in human not evaluated by our study. Nevertheless, we observed that CXCR4, the receptor for CXCL12, was expressed by alveolar fibrocytes, in agreement with different studies suggesting the implication of this signaling pathway in lung diseases (12, 13). To progress on the mechanism involved in lung recruitment of fibrocytes, it would be interesting to determine and compare the kinetics and the levels of fibrocyte in both blood and BAL. To our knowledge, only circulating fibrocytes have been evaluated as yet in a group of ten patients with ARDS included as a control group in a study on idiopathic pulmonary fibrosis (14). In that study, circulating fibrocytes remained moderately increased in patients with ARDS, amounting to 2.13% ± 0.63% of blood mononuclear cells (means±SEM) (14). Circulating fibrocytes might not reflect the pool of recruited progenitors. Indeed, different studies suggest that a major part of fibrocytes detected at the site of injury could derive from a subpopulation of CD14+ peripheral blood mononuclear cells (8, 29) either after stimulation through the CCL2/

CCR2 signaling pathway (30) or in the presence of Th2 cytokines (31). The positive correlation between BAL fibrocyte and mononuclear cell percentages in our study as well as animal studies suggests such a phenomenon. In an experimental model of lung injury, whereas circulating fibrocytes seem moderately increased, the lung fibrocyte counts increase dramatically at the same time after injury (9). Therefore, to evaluate alveolar fibrocytes rather than circulating fibrocyte counts might be more relevant.

The exact role of fibrocytes in lung repair remains uncertain, but these cells have the ability to differentiate into fibroblasts, to produce extracellular matrix, to enhance local mesenchymal population directly or indirectly through the production of TGF-β1 (32), and to regulate the local immune response (8). Although we confirmed that BALF hepatocyte growth factor, TGF-β1, and collagen 1 concentrations were elevated in patients with ALI/ARDS as previously reported (25, 33), we did not find any correlation between the percentage of alveolar fibrocytes and these factors involved in tissue repair. Different studies suggest that fibrocytes are necessary for wound healing, but their excessive recruitment or differentiation could be a key event in fibrotic diseases (8, 14). To date, it remains unclear if the increase of pulmonary fibrocyte during ALI/ARDS is only a marker of lung injury severity or an unsuitable repair process contributing to outcome. Therefore, the functions of fibrocytes during ALI must be further specified before considering the modulation of this cell pathway as a therapeutic option.

It should be noticed that the cutoff level of 6% for fibrocyte in BAL was determined using the receiver operating characteristics curves for mortality at day 28 before performing the Cox proportional hazard model on the same data and not from an external study. BAL fibrocytes as a continuous variable is associated with the outcome as a continuous variable, but further validation is necessary to assess the best cutoff to use for an impact on clinical strategy. Nevertheless, our results identify the BAL fibrocyte percentage as an independent predictor of mortality in patients with ALI/ARDS. Our analysis demonstrates the additive prognostic value of BAL fibrocyte percentage to clinical factors such as age, comorbidity, or organ failure, already known as important predictors of mortality (26, 34). Otherwise, the protocol that we used to determine the BAL fibrocyte percentage can easily be adapted to the clinical setting because flow cytometry results can be provided in <5 hrs after the procedure. After validation on a second cohort of patients, this biologic marker could be useful in clinical strategy to differentiate groups of patients at highest risk for adverse clinical outcomes. In addition to its potential clinical use in stratification of patients for enrollment in clinical trials, this biomarker may help elucidate mechanisms of fibroproliferation during ALI/ARDS and may be valuable in designing new therapies for ALI/ARDS.

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